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Effects of Increased Dosages of the *Mycoplasma gallisepticum* Vaccine MYCOVAC-L® in Layer Chickens Subsequently Challenged with Virulent *M. gallisepticum*: Egg Production and Serologic Response

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SUMMARY. Ten-week-old Hy-Line Commercial W-36 pullets were spray-vaccinated with MYCOVAC-L® at the manufacturer's recommended dosage (1×) or at 15 times that rate (15×). At 22 or 45 wk of age, subsets of 1×- and 15×-vaccinated pullets were challenged with the virulent *Mycoplasma gallisepticum* (MG) strain R_{low}. Percent hen-day egg production was determined through week 55. Analyses for treatment effects on overall (22–56 wk) percent hen-day egg production revealed no significant differences between nonchallenged 1× and nonchallenged 15× MYCOVAC-L® treatments. Among 1× MYCOVAC-L®–vaccinated groups, R_{low} challenge at 45 wk corresponded to significantly ($P \leq 0.01$) lower overall egg production compared with the unchallenged 1×-vaccinated control (70.88% *vs.* 79.15%, respectively). Conversely, at the 15× MYCOVAC-L® dosage level, overall egg production was not significantly affected by virulent MG challenge at 45 wk compared with its unchallenged counterpart (84.09% *vs.* 81.03%, respectively) and could indicate increased protection from virulent MG challenge. Serologic monitoring indicated the virulent MG challenge was consistently (100%) associated with seroconversion. Comparisons among the nonchallenged experimental treatments indicated that vaccinations at the 15× MYCOVAC-L® dosage rate were associated with a greater seroconversion rate at weeks 21, 27, and 44, but not at week 50.

RESUMEN. Efecto de dosis aumentadas de la vacuna contra *Mycoplasma gallisepticum* MYCOVAC-L® en ponedoras subsecuentemente desafiadas con *M. gallisepticum* virulento: producción de huevos y respuesta serológica.

Pollonas comerciales Hy-line W-36 de 10 semanas de edad fueron vacunadas con MYCOVAC-L® a la dosis recomendada por el fabricante (una dosis) o a 15 veces esa dosis. A las 22 ó 45 semanas de edad subgrupos de aves vacunadas se desafiaron con la cepa virulenta R_{low} de *Mycoplasma gallisepticum*. Se determinó el porcentaje diario de producción de huevos por ave hasta la semana 55. El análisis de los efectos de los tratamientos sobre la producción total de huevos por ave no reveló diferencias significativas entre las aves no desafiadas vacunadas con una o con 15 dosis de MYCOVAC-L®. En los grupos vacunados con una dosis de MYCOVAC-L® el desafío a las 45 semanas con la cepa R_{low} generó una producción de huevos significativamente menor ($P \leq 0.01$) en comparación con los controles no desafiados vacunados con una dosis (70.88% *vs.* 79.15%, respectivamente). Contrariamente, a la dosis 15 veces mayor de MYCOVAC-L®, la producción total de huevos no se vió afectada significativamente por el desafío virulento de *Mycoplasma gallisepticum* de las 45 semanas en comparación con su contraparte no desafiada (84.09% *vs.* 81.3%, respectivamente), esto puede indicar una mayor protección contra el desafío virulento con *Mycoplasma gallisepticum*. El seguimiento serológico indicó que el *Mycoplasma gallisepticum* virulento estuvo asociado consistentemente con seroconversión (100%). Las comparaciones entre los tratamientos experimentales no desafiados indicaron que las vacunaciones a la dosis 15 veces mayor están asociadas con mayor seroconversión a las 21, 27 y 44 semanas pero no a la semana 50.

Key words: *Mycoplasma gallisepticum*, vaccination, vaccine, MYCOVAC-L®, live vaccine, egg production

Abbreviations: ELISA = enzyme-linked immunosorbent assay; MG = *Mycoplasma gallisepticum*; MS = *Mycoplasma synoviae*; NPIP = National Poultry Improvement Plan; SPA = serum plate agglutination

Mycoplasma gallisepticum (MG) is the most economically significant mycoplasma species affecting both meat- and egg-type poultry (23) and is frequently encountered within poultry industries worldwide (21,33). In addition to increased mortality, increased carcass downgrades, and decreased feed efficiency, MG infections can reduce egg production in layers and broiler breeder chickens by 10%–20% (4). Furthermore, MG infections have been associated with reduced egg size (7).

Strains of the avian pathogen vary widely in virulence and in the associated serologic response (30) and can be associated with both acute and chronic avian diseases (10). Furthermore, MG has been isolated from the mucosal membranes of the respiratory tract and the urogenital tract (32), the eye (29), and the brain (10) and can be associated with diseases at multiple physiologic sites (21,36). MG, singly, can cause respiratory disease in turkeys. Respiratory disease in chickens, however, appears to result from the interactions of MG and respiratory viruses, *Escherichia coli*, or both, and the resulting disorder could be influenced by multiple environmental factors (17,23).

Effective control of MG infections is hindered by the organism's inherent ability to evade the host's immune system. Like other *Mycoplasma* species and pathogens of other genera, MG strains have the ability to change the expression of surface antigens and thereby to alter the ''antigenic profile'' presented to the host's immune system (2). In addition, immune responses to MG are not well understood. Furthermore, complications toward MG control include the organism's ability to transmit both vertically and horizontally and survive outside the host (11) and the lack of rapid and specific means of detection that differentiates field and vaccine strains.

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MG control strategies commonly used within the poultry industry include intense biosecurity and biosurveillance *via* serologic testing, MG isolation, and DNA-based detection methods (21). Within the layer industry, live attenuated MG vaccines have been approved and are widely used to minimize the effect of infections by virulent MG. These vaccines include FVAX-MG® (Schering-Plough Animal Health, Omaha, NE), MYCOVAC-L® (Intervet Inc., Millsboro, DE), and MG vaccine® (Merial Select Laboratories, Gainesville, GA), and each strain has been shown to effectively reduce losses associated with MG field strain challenge (9,12,37). Characteristics that distinguish these vaccines include pathogenicity, transmissibility, protection afforded (5), serologic response, and the recommended application/vaccination route (16). To date, the means by which these live vaccines provide protection is largely unknown (15).

Previous findings have shown that subjects vaccinated with MYCOVAC-L® according to manufacturer's recommendations demonstrate minimal pathology (e.g., respiratory distress, lesions) (22) and negligible antibody response (1,13,22) and have low vaccine persistence rates (1,22) that could have implications on protection afforded by the vaccine. Preliminary results from a laboratory-associated study indicated that both the *in vivo* persistence of MYCOVAC-L® and the associated serologic responses were enhanced with higher vaccination doses (data not shown). Therefore, the following study was performed to further investigate the effect of increased dosages of MYCOVAC-L® on egg production of commercial layers subsequently challenged with the commonly used virulent MG challenge strain R_{low} (15). Egg production, egg size, and serologic response were selected to assess treatment effects.

MATERIAL AND METHODS

Animals and housing. Hy-Line Commercial W-36 laying hens ($n = 176$) used in this trial originated from a commercial source certified MG- and *Mycoplasma synoviae* (MS)–free under National Poultry Improvement Plan (NPIP) guidelines (25). The chicks were obtained at 1 day of age and were placed on clean pine shavings in a conventional house. Artificial lighting was continually provided for the initial 48 hr and was subsequently reduced to 10 hr/day, which was maintained through 10 wk of age (5). Diets were formulated to meet or exceed National Research Council guidelines (26), and feed and water were provided *ad libitum* for the duration of the study. At 5 wk of age, the pullets were vaccinated for infectious bursal disease, Newcastle disease, and infectious bronchitis and sampled for exposure to MG and MS *via* serum plate agglutination (SPA) and MG culture of choanal cleft swabs (5). At 10 wk of age, the experimental subjects were transported to an environmentally controlled disease isolation facility containing 16 negative-pressure fiberglass biological units within a single room (8). The upper beaks of the pullets were trimmed to prevent cannibalism, and the birds were randomly placed in the biological isolation units at a rate of 11 pullets/unit. Within the facility, the temperature was maintained at 23 C throughout the study and through 18 wk of age, lighting was provided at 10 hr/day. At approximately 14 wk of age, one bird per unit was euthanized by cervical dislocation to achieve a final stocking rate of 10 birds/unit. At 18 wk of age, lighting was increased 15 min/wk until a threshold of 16 hr 15 min was attained. Throughout the experiment, care of the hens was consistent with guidelines approved by the Institutional of Animal Care and Use Committee.

Experimental design. Each biological isolation unit was assigned to one of six treatments. Treatment designation was made to prevent cross-contamination across the various treatment groups, with two or more replicates per treatment.

Treatment 1. MYCOVAC-L® 10 wk vaccination at the manufacturer's recommended dosage rate (1×); four replicates.

Treatment 2. MYCOVAC-L® 10 wk vaccination at the manufacturer's recommended dosage rate (1×) with virulent MG challenge at 22 wk; two replicates.

Treatment 3. MYCOVAC-L® 10 wk vaccination manufacturer's recommended dosage rate (1×) with virulent MG challenge at 45 wk; two replicates.

Treatment 4. MYCOVAC-L® 10 wk vaccination at 15× the manufacturer's recommended dosage rate (15×); four replicates.

Treatment 5. MYCOVAC-L® 10 wk vaccination at 15× the manufacturer's recommended dosage rate (15×) with virulent MG challenge at 22 wk; two replicates.

Treatment 6. MYCOVAC-L® 10 wk vaccination at 15× the manufacturer's recommended dosage rate (15×) with virulent MG challenge at 45 wk; two replicates.

MG vaccination. An average MYCOVAC-L® vaccine titer of 2.1×10^8 color-changing units/ml was estimated by sampling two vials originating from the same lot as that used in the study. The vaccine was suspended at 1× and 15× concentrations in nonchlorinated water (reverse osmosis–purified, 18.2 MΩ) according to manufacturer's recommendations. Vaccine application occurred *via* a fine spray at the 1× or 15× dosage rate to pullets contained in coops.

MG challenge. For virulent MG challenge, *M. gallisepticum* strain R_{low} (13 passages) was kindly provided by Dr. Steven Geary (University of Connecticut). R_{low} was cultured at 37 C in FMS media (35) supplemented with 35 ml of yeast extract solution per liter (#18180-059, Invitrogen/Gibco, Carlsbad, CA). On color indicator (phenol red) change (>18 hr), 40 μl of culture was inoculated intraocularly.

MG diagnostics. Subjects were bled at weeks 21, 27, 44, and 50 from the cutanea ulnae (wing) vein. Serum components were separated by sedimentation of the red blood cells, and all sera were tested for antibodies to MG by SPA analysis adapted from Yoder (39). Briefly, 25 μl of commercially obtained antigen was mixed with 25 μl of serum on a ruled glass plate. The mixture was rotated for approximately 3 min on a mechanical mixer, and degree of agglutination was primarily performed as per Stanley *et al*. (34), except for the use of an agglutination scale ranging from 0 (no agglutination) to 3 (strong agglutination). For determination of percent flock seroconversion, all positive SPA scores (1, 2, or 3) were grouped and compared with total birds of each particular treatment.

Egg production/size. Throughout the study (weeks 20–55), eggs were collected daily, and egg production was recorded on a biological isolation unit basis. Eggs collected between weeks 22 and 56 were used to calculate the overall percent hen-day egg production. During weeks 27 and 50 (5 wk post-R_{low} challenge at 22 and 45 wk), all eggs produced within the biological isolation units housing 1×- and 15× MYCOVAC-L®–vaccinated and subsequently R_{low}-challenged (22 or 45 wk) hens were collected and individually weighed, and results were recorded. Results were compared with eggs produced in two similarly vaccinated nonchallenged control replicates.

Statistical analyses. Data were statistically evaluated as a completely randomized design with a 3×2 factorial arrangement of treatments as previously described. Each treatment was represented by at least two replicate biological isolation units, and the biological isolation unit was considered the experimental unit. Data were analyzed by analyses of variance according to the general linear model (31). Means were separated by contrast statements. Data were considered statistically significant at $P \leq 0.05$.

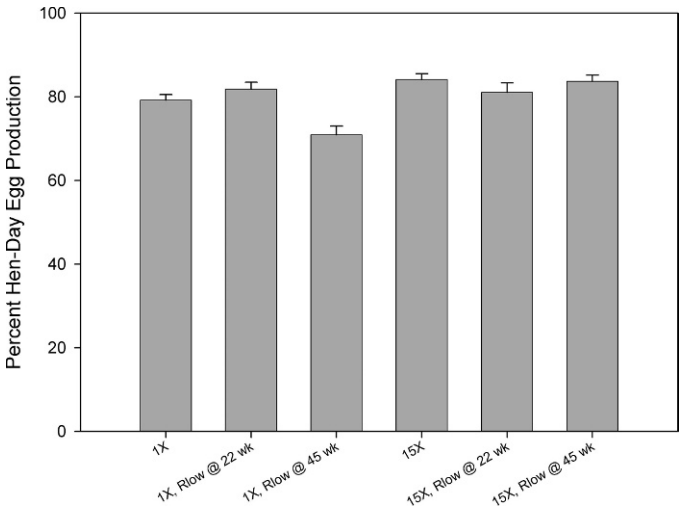

Fig. 1. Overall percent hen-day egg production of 1× and 15× MYCOVAC-L®–vaccinated layers with and without R_{low} at 22 or 45 wk. Mean value ± SEM is represented.

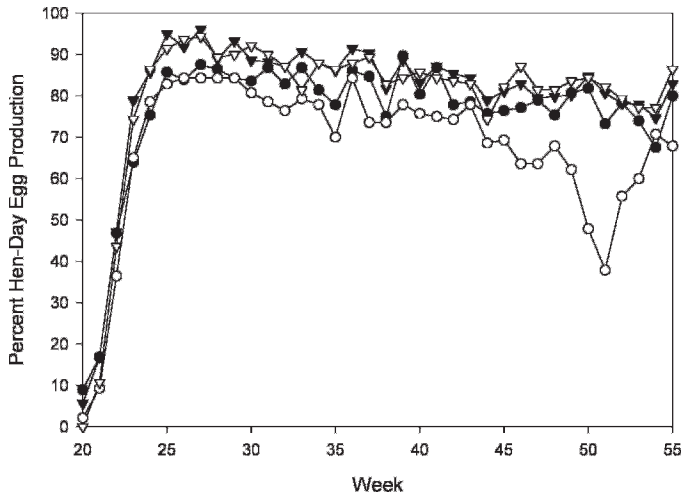

Fig. 2. Weekly percent hen-day egg production of 1× and 15× MYCOVAC-L®–vaccinated layers with and without R_{low} challenge at 45 wk. (●) Nonchallenged 1× MYCOVAC-L®–vaccinated layers,(○) R_{low}-challenged 1× MYCOVAC-L®–vaccinated layers, (▲) nonchallenged 15× MYCOVAC-L®–vaccinated layers, (▼) R_{low}-challenged 15× MYCOVAC-L®–vaccinated layers. Values represent means of two (R_{low}-challenged 1× and 15× MYCOVAC-L®–vaccinated layers) or four (nonchallenged 1× and 15× MYCOVAC-L®–vaccinated layers).

RESULTS

Egg production. Overall egg production as represented by percent hen-day egg production for study-associated treatments is shown in Fig. 1. Percent hen-day egg production within the 1× MYCOVAC-L® treatment group was not affected by R_{low} challenge at 22 wk. However, within the 1× treatment groups, R_{low} challenge at 45 wk was associated with lower ($P \leq 0.05$) egg production compared with the unchallenged control (70.88% *vs.* 79.15%, respectively). Within the 15× MYCOVAC-L®–vaccinated groups, percent hen-day egg production did not differ when comparing the nonchallenged controls with birds challenged at 45 wk with R_{low}. However, 15× MYCOVAC-L®–treated birds challenged at 22 wk demonstrated lower ($P \leq 0.05$) hen-day egg production than their nonchallenged counterparts, although the difference was slight (81.03% *vs.* 84.1%, respectively). Overall hen-day egg production by nonchallenged hens vaccinated with 1× or 15× MYCOVAC-L® was not statistically different (79.15% *vs.* 84.1%, respectively). When comparing egg production of hens challenged at 22 wk with R_{low}, the birds receiving the 1× dosage of MYCOVAC-L® produced slightly more eggs ($P \leq 0.05$) than similarly challenged 15×-treated birds (81.79% *vs.* 81.03%, respectively). A larger difference was determined among birds challenged with R_{low} at 45 wk, wherein birds receiving the 15× dosage of MYCOVAC-L® produced significantly ($P \leq 0.05$) more eggs than their 1×-dosed counterparts (83.68% *vs.* 70.88%, respectively). Fig. 2 depicts the weekly percent hen-day egg production for nonchallenged 1× and 15× MYCOVAC-L® and their week 45 R_{low}-challenged counterparts.

Egg size. Mean egg size (weight) as determined 5 wk post-R_{low} challenge (weeks 27 and 50) is shown as Table 1. Data collected during week 27 (5 wk post-R_{low} challenge at 22 wk) indicated that mean weights of eggs produced early in the laying cycle were not affected by dosage level or by R_{low} challenge because no significant differences were observed (data not shown). Within this sampling, however, eggs from nonchallenged vaccinated birds were numerically larger than those from identically dosed and R_{low}-challenged layers and the 1× MYCOVAC-L®–vaccinated/R_{low}-challenged birds did produce the smallest eggs. Among eggs collected during week 50 (5 wk post-R_{low} challenge at 45 wk), contrasts revealed slight but significant differences ($P \leq 0.05$) between eggs produced by the 1× MYCOVAC-L®–dosed layers challenged with R_{low} at 22 wk and their nonchallenged counterparts (59.46 *vs.* 60.33 g; data not shown). Within the 15× MYCOVAC-L® treatment groups, eggs produced by nonchallenged layers were slightly, yet significantly, larger ($P \leq 0.0004$) than those from birds challenged with R_{low} at 45 wk. Comparisons among similarly challenged treatment groups varying only in dosage rate showed that eggs produced during week 50 by 1× MYCOVAC-L®/R_{low}–challenged (45 wk) birds were statistically ($P = 0.0005$) larger than their 15× counterparts (60.83 *vs.* 58.78 g, respectively).

Serology. The serologic data for the experiment are summarized in Table 2. As measured at 27 or 50 wk (5 wk postchallenge), the MYCOVAC-L®–vaccinated chickens did not seroconvert strongly or consistently unless challenged with the R_{low} strain of MG, which was associated with 100% seroconversion or 100% reactors. At 21 wk, SPA analyses demonstrated an 85% seroconversion (50% + 35%) rate among the 15× MYCOVAC-L®–vaccinated birds, whereas corresponding tests of the 1×-vaccinated birds revealed no seroconversion. Sampling at 27 wk yielded seroconversion rates among nonchallenged birds of 20% and 25% for the 1×- and 15×-vaccinated birds, respectively. Similar to prechallenge SPA analysis at 21 wk, tests at 45 wk demonstrated increased seroconversion rates for 15×-vaccinated birds compared with those treated at the 1× MYCOVAC-L® dosage (35% *vs.* 0%). Interestingly, SPA analyses of non–R_{low}-challenged birds at 50 wk revealed increased seroconversion among the 1×-vaccinated birds (50.0% *vs.* 31.6% for the 1× and 15× treatment groups, respectively).

DISCUSSION

Live MG vaccines have been assessed on the basis of their safety to the vaccinated host and on the protection that they afford that host from virulent MG strains. Safety issues addressed are basically limited to assessment of vaccine strain transmissibility and virulence (3,27) but also include appraisal of any vaccine-related effects on layer production (5,6). Specifically, the efficacies of live vaccines for control of MG infections have been assessed by their abilities to displace field

Table 1. Influence of 1× or 15× MYCOVAC-L® vaccination at 10 wk with or without R_{low} challenge at 22 or 45 wk on egg weight.

	Egg weight (g)					
	Week 27			Week 50		
Treatment	*n*	Mean	SEM	*n*	Mean	SEM
1×, Nonchallenged	83	52.64	0.429	215	60.33	0.237
1×, R_{low} at 22 wk	88	52.40	0.344	109	59.46	0.495
1×, R_{low} at 45 wk	—	—	—	54	60.83	0.373
15×, Nonchallenged	95	53.37	0.259	231	60.24	0.240
15×, R_{low} at 22 wk	87	53.36	0.405	97	59.17	0.301
15×, R_{low} at 45 wk	—	—	—	113	58.78	0.330

Contrasts	*P*	*P*
Nonchallenged,1× *vs.* 15×[A]	0.151	0.788
R_{low} at 22 wk, 1× *vs.* 15×[B]	0.051	0.564
R_{low} at 45 wk, 1× *vs.* 15×[C]	—	0.0004

[A]Comparison of nonchallenged layers vaccinated with 1× and 15× MYCOVAC-L®.
[B]Comparison of 1× and 15× MYCOVAC-L®–vaccinated layers challenged with R_{low} at 22 wk.
[C]Comparison of 1× and 15× MYCOVAC-L®–vaccinated layers challenged with R_{low} at 45 wk.

strains (18,20), protect against MG-associated lesions (12), and minimize MG-associated drops in production (9). In addition, *in vivo* persistence of these vaccines has been implicated as a factor affecting vaccine efficacy (14). With a variety of vaccines, research has indicated that vaccination levels or dosages can affect the protection afforded (19,24,38); however, research pertaining to this factor is limited regarding live MG vaccines. In 1984, Lam and Lin (19) determined that vaccination with increased doses of a temperature-sensitive MG mutant increased protection against air sac lesions. Whithear *et al.* (38) in 1990 demonstrated that 10-fold dosages of ts-11 applied *via* eye-drop were associated with an increased immune response compared with that of the layers vaccinated at the lower 1× level and that this immune response was prolonged compared with that associated with the 1×-counterpart. However, the increased vaccination load was also associated with decreased egg production following virulent MG challenge. Furthermore, when increased dosages (0.1×–5×) applied *via* aerosol application were investigated, a serologic dose response was not apparent (38).

In this study, an elevated (15×) MYCOVAC-L® vaccination was compared with the manufacturer's recommended rate (1×) on layers subsequently challenged with a virulent strain of MG (R_{low}) early (22 wk) or late (45 wk) in their lay cycle. Measured effects included treatment-associated daily/overall egg production, egg size (weight) determination, and hen seroconversion rates.

Previous research has suggested that MG infections originating around 45 wk can have a more significant effect on layer production when compared with layers infected at an earlier age. In that work (7), researchers reported that infections at this time by the moderately virulent F strain of MG (an attenuated vaccine strain) can reduce overall egg production by approximately 5.8%. In this study, overall percent hen-day egg production comparisons revealed that, among layers treated at the manufacturer's recommended rate (1×), virulent MG challenge at 45 wk was associated with decreased overall egg production (~10%) compared with that of their nonchallenged and 22 wk R_{low}-challenged counterparts. This drop in overall egg production was, however, not evident with 15× MYCOVAC-L®–vaccinated and identically challenged (R_{low} at 45 wk) layers.

Fig. 2 provides a more detailed view of the effect of the virulent MG challenge at 45 wk and reveals the drop in egg production associated with the challenge of the 1× MYCOVAC-L®–vaccinated birds. This figure also shows that no significant production losses were apparent with either the identically challenged 15× MYCOVAC-L® vaccination or with the nonchallenged 1× and 15× vaccinations. Significant egg production losses of this magnitude are not uncommon, in that losses of >50% have previously been reported with MG-infected layers (13).

Interestingly, egg size (weight) analyses, revealed that even though the 15× MYCOVAC-L®–treated and 45 wk MG-challenged birds produced more eggs, they were smaller than those produced by their 1×-treated counterparts (Table 1). However, large differences in egg size (weight) were not observed, and mean sizes of all eggs from the 50 wk collection independent of treatment were larger than the standard large egg size of 56.7 g (28).

Percent seroconversion or the percentage of reactors differed among treatments and according to the sampling schedule. One might predict that the higher dosage of MYCOVAC-L® would be associated with a greater number of seroconverting or reacting birds, and this relationship has indeed been previously shown (38). In the study described herein, the dose–response relationship is apparent when comparing the number of reactors found among the

Table 2. Serologic responses of chickens after vaccination with MYCOVAC-L® at 10 wk with 1× or 15× the recommended dosage with or without R_{low} challenge at 22 or 45 wk.

		Percent reactors (mean SPA[A] score)			
		22 wk		45 wk	
Vaccine dosage	R_{low} challenge	21 wk (1 wk prechallenge)	27 wk (5 wk postchallenge)	44 wk (1 wk prechallenge)	50 wk (5 wk postchallenge)
1×	No	0 (0)	20 (0.20)	0 (0)	50 (0.50)
1×	Yes	0 (0)	100 (1.0)	0 (0)	100 (1.05)
15×	No	50 (0.50)	25 (0.25)	10 (0.10)	31.6 (0.30)
15×	Yes	35 (0.35)	100 (1.05)	25 (0.25)	100 (1.00)

[A]Serum plate agglutination.

nonchallenged 15× MYCOVAC-L® treatments for the 21, 27, and 44 wk samples. However, SPA analyses at 50 wk identified more reactors among the 1× MYCOVAC-L®–vaccinated and nonchallenged birds compared with their 15×-vaccinated counterparts. In regards to the study described herein, the small sample size, the subjective nature of the SPA test, and the experiment-associated low SPA grades (which were approaching detection limits) must be considered. Furthermore, the importance of the seroconversion rate as predicted by SPA could be further limited because previously researchers have questioned the relationship of seroconversion and vaccine efficacy (20,27).

The data presented in this paper indicate that increased dosages of MYCOVAC-L® might enhance protection from egg production losses normally associated with MG infection. Although a dosage 15 times (15×) that recommended by the manufacturer was used in this study, the authors do not advocate the use of this extreme dosage, which might be cost limiting. However, the research does indicate that further work is necessary to determine the minimal dosage rate to effect maximal protection and any deleterious consequences of these larger doses.

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